5/12/88

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAY 12 1988

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Naled - Three-Week Inhalation Study, Final Report
Submitted Under Accession No. 400872-01
EPA Registration No. 239-1633

TB Project No.: 8-0455
Caswell No.: 586

FROM: Irving Mauer, Ph.D. 05/11/88
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: John T. Tice/Dan Peacock, PM Team 16
Insecticide-Rodenticide Branch
Registration Division (TS-767C)

THRU: Judith W. Hauswirth, Ph.D., Head 5/12/88
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Chevron Chemical Company 5/12/88
Ortho Agricultural Chemicals Division
Richmond, CA

Request:

Review and evaluate the following study, originally submitted in response to the Data Call-In Notice generated from the Naled Registration Standard, dated June 1983:

SOCAL 2334: Three-Week Aerosol Inhalation Toxicology Study of Chevron Naled Technical (SX-1554) in Rats, performed at the Chevron Environmental Health Center (CEHC), Richmond, CA, Study No. S-2457, Final Report dated December 11, 1986 (EPA Accession No. 400872-01).

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Background:

A set of preliminary data* from this range-finding study has already been submitted (May 9, 1985), and briefly reviewed (memorandum: Mauer to Miller/Otakie, dated July 24, 1985, with attached DATA REVIEW, TB Document No. 004580, appended to this memorandum). In summary, these preliminary data represented only ChE effects and chamber concentrations; clinical observations, body weights, food consumption, hematology, serum chemistry, organ weights and pathology were to be submitted later (the present Final Report). The preliminary report was considered Supplementary Data, since the LDT, 3.4 ug/L (actual concentration), was an effect level, depressing RBC-AChE by 26 percent, plasma-ChE by 33 percent, and brain enzyme levels by 23 to 28 percent.

The results of this preliminary study were to be used to select doses for the subsequent 13-week study in rats with Chevron Naled Technical (SOCAL 2400),** which has also been reviewed (Data Review, attached to memorandum: Mauer to Miller/Otakie, March 19, 1987--TB Document No. 005784), and judged CORE-MINIMUM data. In this adequate subchronic study, groups of 12 young adult Fischer-344 males and females each were exposed 6 hours/day, 5 days/week for 13 weeks to nominal concentrations of 0 (filtered air), 0.2, 1.2, and 6 ug/L test material. Additional groups of control and high-dose animals (10/sex/group) were allowed to recover for a 6-week nontreatment period ("satellite" groups). Briefly, the results of this 13-week assay were:

1. Routine sampling of chamber atmospheres revealed mean daily concentrations of naled were actually 0.23, 1.29, and 5.80 ug/L for the low-, mid-, and high-dose groups, respectively; concentrations of bromodichloroacetaldehyde (BDCA) (a hydrolysis product) = 0.18, 0.31, and 0.93 ug/mL, respectively; and concentrations of DDVP (considered by the registrant as "a minor constituent of the technical)" = 0.01, 0.05, and 0.09 ug/L, respectively.

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*Three-Week Aerosol Inhalation Toxicity Study of Chevron Naled Technical in Rats--Preliminary Data Release: SOCAL 2334, March 7, 1985, S-2457, CEHC, May 9, 1985 (EPA Accession No. 257963.

**Thirteen-Week Aerosol Inhalation Toxicology Study of Chevron Naled Technical (SX-1655) in Rats. SOCAL 2400, CEHC Study No. S-2438, dated August 26, 1986 (EPA Accession Nos. 265678, 265679, and 265680).

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2. The NOEL for inhibition of circulating cholinesterase (ChEI) = 0.23 ug/L (the LDT); the NOEL for tissue-bound (brain) AChEI = 1.29 ug/L; and the systemic NOEL for clinical signs of ChEI = 1.79 ug/L.

3. There were no treatment-related hematological, biochemical, pathological, organ weight, or microscopic changes.

TB Conclusions (on the Range-Finder, SOCAL 2334--Detailed TB Data Review Attached):

The target concentrations generated = 0, 4, 8, and 16 ug/mL.

Actual concentrations measured = 0, 3.4, 7.2, and 12.1 ug/mL.

No NOEL found, the LDT producing ChE inhibition (RBC, plasma, and brain), as well as nasal epithelial lesions.

CORE-SUPPLEMENTARY DATA

Attachments

REVIEWER

UNITED STATES ENVIRONMENTAL PRCTECTION AGENCY
WASHINGTON, D C 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

JUL 24 1985

MEMORANDUM

SUBJECT: Naled RS - Data submitted May 9, 1985, in Response to DCI (21-day Rat Inhalation) under Accession No. 257963. EPA Registration No. 239-1633

Caswell No. 586

FROM: Irving Mauer Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769)

TO: William Miller/G. Otakie, PM-16
Registration Division (TS-767)

THRU: Jane E. Harris, Ph.D., Section Head
Section VI
Toxicology Branch
Hazard Evaluation Division (TS-769)

Registrant

Chevron Chemical, Richmond CA.

Action Requested

Review and evaluate the following study, submitted May 9, 1985:

Three-Week Aerosol Inhalation Toxicity Study of Chevron Naled Technical in Rats--Preliminary Data Release: SOCAL 2334, March 7, 1985, S-2457, performed at the Chevron Environmental Health and Toxicology Center, Richmond CA.

TB Conclusion

Core Supplementary Data. (See below, and attached TOXICOLOGY BRANCH: DATA REVIEW.)

Background

These preliminary data were submitted as a range-finding study in order to select dose levels to be used in a 90-day subchronic inhalation study to be scheduled at a later date. According to the registrant, the purpose of this submission was ". . . to present data on the cholinesterase effects and chamber concentrations. [Further] A report summarizing the other data collected during this study will be issued at a later date." (from Section II of Report S-2457).

It should be noted that both an acute inhalation LC_{50} study in the rat as well as a 90-day inhalation study are required to be submitted under FIFRA section 3(c)(2)(B) [NALED Pesticide Registration Standard, issued June 1983 - Table A, Section 158.135 Toxicology]. The present report satisfies neither of these data requirements.

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TOXICOLOGY BRANCH DATA REVIEW

CHEMICAL: Naled

Caswell: 586
EPA Chem.#: 034401

STUDY TYPE: Subchronic (21-day Range-Finding) Inhalation - Rat

CITATION: "Three-week Aerosol Inhalation Toxicity Study of Chevron Naled Technical in Rats - Preliminary Data Release Socal 2334."

ACCESSION No./MRID No.: 257963/NA

SPONSOR/TESTING LAB: Chevron/Environmental Health and Toxicology (Chevron)

STUDY NO./DATE: S-2457/March 7, 1985

TEST MATERIAL: Naled technical, SX-1554 (89.3% ai), a pale yellow viscous liquid

PROCEDURES:

Four groups of young adult (60-day old) male and female Fischer-344:CDF (F-344)/Crl Br rats were exposed to an aerosol of undiluted test material at nominal concentrations of 0 (filtered air only), 4, 8, and 16 ug/L for 6 hours/day for a total of 15 exposures over a 21-day period. Actual chamber concentrations of naled and bromodichloroacetaldehyde (BDCA, a hydrolysis product of naled) were determined hourly throughout the exposure period, while dichlorovos (DDVP), a minor constituent of technical naled, was determined once a week. Particle size was monitored weekly. Red blood cell and plasma cholinesterase activities were determined after the fifth exposure (orbital sinus samples) and at sacrifice (15 exposures), at which time half-brains were also analyzed for cholinesterase activity. At necropsy, selected organs were weighed (lungs, liver, kidneys, adrenals, and brain) and a roster of 27 organs and tissues as well as any gross lesions examined for pathological changes; nasal passages, trachea, lungs, and testicles were submitted for histopathological examination.

RESULTS:

The overall mean chamber concentrations based on chemical analysis of the chamber atmosphere during the course of the 3-week exposures were given as 3.4, 7.2, and 12.1 ug/L (table 1 of

report). Significant dose-related depressions of circulating (greater after 15 exposures) as well as brain cholinesterase activities were noted at all concentrations of naled in both males and females (tables 2 through 5 of report). Compared to control values, mean percent inhibition of RBC-AChE after five exposures ranged from 26 percent (low-dose females) to 84 percent (high-dose females), while mean plasma ChE inhibition ranged from 33 percent (low-dose males) to 89 percent (high-dose females). At termination (15 exposures), low-dose male and female RCB-AChE registered 58 percent and 43 percent inhibition, respectively, while high-dose values were 94 percent and 93 percent; plasma values did not change at any dose level. Brain cholinesterase activity was significantly depressed ($p \leq 0.01$) at all doses in both sexes, ranging from 23 to 28 percent at the LDT to over 60 percent in high-dose animals. The authors noted that the animals may have been exposed dermally as well as orally (due to whole body exposure), but could not estimate the amount of test substance absorbed by these routes. [Clinical observations, body weights, food consumption, hematology, serum chemistry, organ weights, and pathology are to be submitted later in the final report.]

TB EVALUATION: NOEL for cholinesterase inhibition is less than 3.4 ug/L (actual)

CORE SUPPLEMENTARY DATA.

NOTE: According to test data gaps defined in the Naled Registration Standard, both acute LC_{50} and 90-day inhalation NOEL values are required to be submitted.

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TOXICOLOGY BRANCH: DATA REVIEW

Reviewed By: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division

TB Project: 8-0455

Date: 05/11/88

Through: Judith W. Hauswirth, Ph.D., Head
Section VI, Toxicology Branch
Hazard Evaluation Division

Judith W. Hauswirth 5/12/88

Chemical: Naled

Caswell: 586
EPA Chem: 034401

Study Type: Three-Week Inhalation--Rat (Range-Finder)

Citation: SOCAL 2334--Aerosol Inhalation Toxicity Study of Chevron Naled Technical (SX-1554) in Rats

Accession No.: 400872-01

MRID No.: N/A

Sponsor: Chevron Chemical Company
Ortho Agricultural Chemicals Division
Richmond, CA

Testing Lab: Chevron Environmental Health Center (CEHC)
Richmond, CA

Study No.: S-2457

Study Date: December 11, 1986

TB Conclusions/Evaluation: Core-Supplementary Data (Range-Finding Study)

Target Doses Tested: 0, 4, 8, 16 ug/mL (concentrations actually found = 0, 3.4, 7.2, 12.1 ug/mL). No NOEL, the LDT producing cholinesterase inhibition (RBC, plasma, brain); nasal epithelial lesions.

DETAILED REVIEW

Test Material:

Naled technical, Lot No. SX-1554, 90% ai (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), plus other constituents:

[redacted]

Clear to pale viscous liquid, used neat for testing.

Test Organism:

Young adult (2 months old) male (164 to 223 g) and female (116 to 137 g) Fischer 344 (CDF(F-344)/Crl Br) rats from Charles River Laboratories, Kingston, NY, were allowed to acclimate for 29 days, then randomly assigned to treatment groups (by Chevron's DATATOX WTALOC program) to minimize differences in mean body weights. Each animal was uniquely identified by a combined lettered and numbered ear tag, and a cage card with assignment to a particular test group.

Procedures:

1. Inhalation Chambers - Hazelton Systems (Aberdeen, MD) model H1000 1 m^3 stainless steel/glass chambers were used. The test units were maintained under slightly negative pressure (-1.3 to -1.8 cm water).

2. Aerosol Generation and Delivery - Undiluted test article was nebulized from a continuously stirring 150 mL reservoir, with room air drawn through the nebulizer to sweep the aerosol into a manifold system that delivered it to each of the three test exposure chambers. Naled concentrations delivered to the three chambers were adjusted by changing the pressure differential between chamber and manifold, opening or closing the gate valve connecting each chamber to the manifold, or changing the total air flow to a particular chamber, or by changing the concentration of naled in the manifold.

3. Monitoring Chamber Environments - Chamber supply and exhaust flow, static pressure, temperature, and relative humidity were recorded at the beginning of each exposure and hourly thereafter during each exposure. Aerosol concentrations were monitored in both the chambers as well as the manifold during each exposure.

4. Sampling Chamber Atmospheres - Samples of chamber atmospheres were drawn during each exposure, and analyzed for naled and for BDCA (a hydrolysis product of naled). Selected samples were also analyzed for DDVP (test days 3, 8, and 13).

   A weekly sample from each chamber was analyzed for particle size. Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated for each sample.

5. Animal Exposure - Groups of 10 males and 10 females were exposed to filtered air (control) or aerosols of Naled Technical 6 hours/day, 5 days/week (total of 15 exposures over a 21-day period) at target concentrations of 4, 8, and 16 ug/mL. Animals were sacrificed during the 2-day period following the last exposure (one-half of each sex on study days 21 and 22).

6. Animal Observations - Animals were checked 3 times daily, once before transfer to the exposure chamber, and twice during the 6-hour exposure. Body weights were taken and food consumption measured before the first exposure and weekly thereafter. These parameters were entered into the lab's DATATOX computer system.

   Full ophthalmological examinations on each animal were conducted twice by an independent veterinarian, 26 days prior to the start of the study and on study day 16.

7. Cholinesterase Determinations - Erythrocyte (AChE) and plasma (BChE) cholinesterase enzyme activities from one-half of each group were measured from fresh samples after five exposures (from orbital bleeding), and from the same animals the day after the last exposure (just prior to necropsy). Brain cholinesterase activity (AChE) was determined some time later from one-half the organs frozen during necropsy.

8. Necropsy - On study days 21 and 22, animals were injected ip with sodium pentobarbital, exsanguinated via the abdominal aorta, and the following organs examined for gross changes, resected, and preserved in 10 percent neutral formalin:

Brain	Uterus
Pituitary	Seminal vesicles
Skull (nasal passages)	Aorta (thoracic)
Thyroid/Parathyroid	Skin (with mammary glands)
Thymus	Eyes
Trachea	Esophagus
Lungs	Stomach

Heart	Small intestine (duodenum, jejunum, ileum)
Salivary glands	Large intestine (cecum, colon, rectum)
Liver	Urinary bladder
Spleen	Mesenteric lymph node
Kidneys (both)	Sternum (bone marrow)
Adrenals (both)	Gross lesions
Testes (both)	
Ovaries (both)	
Pancreas	

9. Organ Weights - Fresh wet weights of lungs, liver, brain, adrenals, testes, and kidneys were entered into the DATATOX system, which computed organ-to-body-weight and organ-to-brain-weight ratio using terminal body and whole brain weights obtained on the day of necropsy.

10. Histopathology - Nasal passages, trachea, lungs, testes, and tissues with gross lesions from all animals were processed for microscope slide preparation (by an outside laboratory). After verification of animal-tissue-block count, the slides were evaluated for abnormalities.

11. Clinical Laboratory Studies - The following hematological and serum chemistry parameters were determined from abdominal aorta blood samples from all animals (blood processed by an outside contractor):

Hematology:

Erythrocyte count	Hemoglobin
Total leukocyte count	Mean cell volume
Differential leukocyte count	Mean cell hemoglobin
Platelet count	Mean cell hemoglobin concentration
Hematocrit	

Serum Chemistry:

Calcium	Albumin
Phosphorus	Globulin
Chloride	Albumin/globulin ratio
Sodium	Blood urea nitrogen (BUN)
Potassium	Creatinine
Glucose	BUN/creatinine ratio
Cholesterol	Alkaline phosphatase
Total bilirubin	Alanine aminotransferase (ALT)
Uric acid	Aspartate aminotransferase (AST)
Total protein	Lactate dehydrogenase (LDH)
Triglycerides	Creatinine phosphokinase (CPK) activity

12. Statistical Analysis - Numeric data on body weights, food consumption, cholinesterase activities, clinical chemistry, hematology, organ weights and organ weight ratios, stored in the laboratory's DATATOX system, were analyzed by Bartlett's test for homogeneity of variances, followed by ANOVA. If significant (ANOVA $p \leq 0.05$), individual group comparisons were run with Dunnett's test (2-sided) to identify the statistically significant differences between groups.

Results:

1. Chamber Atmosphere Measurements - During the study, test groups were exposed to the following overall mean concentrations of naled, BDCA and DDVP (Report Table 4; Appendix A):

Exposure Group (Target Conc. ug/L)	Actual Concentration		
	Naled (ug/L)	BDCA (ug/L)	DDVP (ug/L)
Low (4.0)	3.4	0.53	0.08
Mid (8.0)	7.2	0.66	0.13
High (16.0)	12.1	1.0	0.22

Reduction in naled concentration by about 6 percent occurred at the end of a typical 6-hour exposure period (Report Table 5; Appendix A); the authors attributed the loss to hydrolysis of naled to BDCA during the process of generation and/or sampling. [It is evident that the measured concentrations of BDCA in the exposure chambers (0.53 to 1.0 ug/L) are higher than expected from the 0.29 percent content in the SX-1554 formulation.]

Although the concentrations of DDVP measured in the chambers were small, they were proportionately greater, relative to naled, in the LOW and MID chambers, compared to the HIGH chamber. This was attributed by the authors to the volatility of DDVP, leading to preferential vaporization of DDVP during (low-concentration) aerosol generation.

Analysis of particle size distribution (Report Appendices A and B) provided the following mean calculated MMADs and GSDs:

	Concentration		
	Low	Mid	High
MMAD (um)	1.61	2.04	2.18
GSD	1.85	1.91	1.86

2. Clinical Observations

Mortality - No treatment-related deaths were reported. One low-concentration female died on study day 7 following orbital bleeding, attributable by the authors to excessive ether anesthesia.

Toxicity - Signs of clinical toxicity attributable to cholinesterase inhibition were recorded in all exposure groups, in a dose-related manner (Appendix C).

Salivation was common in both mid-dose (8/10 males and 6/10 females) and high-dose animals (10/10 males and 6/10 females), but only occasionally seen at the low dose (1/10 male and 2/10 females). Nasal discharges were recorded in mid- and high-dose groups, but ocular discharges appeared to be common in all groups, including controls (as indicated in the incidence tabulation of Appendix C, in contrast to the text, which stated that such discharges occurred predominantly in high-dose animals).

Abnormal respiratory sounds were common in mid- (9 males and 8 females) and high-dose animals (all 10 males and 10 females), and breathing became visibly labored in all high-dose animals late in the study. In addition, other treatment-related toxicities were recorded in high-dose animals: general weakness (4 females); unkempt appearance (all animals); anogenital discharge (3 males, 4 females); reduced food intake (2 males, 3 females); thinness (5 males, 10 females); reduced fecal output (3 males, 4 females); and corneal opacities (4 females).

The authors stated that "ophthalmologic examination indicated exposure-related increased incidence of corneal edema, iris hyperemia, and keratitis in the eyes of male and female rats in the HIGH concentration group at the end of the exposure period," a summary of which is included in the pathologist's report (Appendix M).

Body Weight - Mean body weight of high-dose males and females was significantly less ($p < 0.01$) than respective controls at all time periods (study days 7, 14, and 21), as was that of mid-dose females ($p < 0.05$) but not males (Report Table 6; Appendix D). These weight changes were considered exposure-related.

Food Consumption - Significantly less food than respective controls was consumed by high-dose males and females ($p < 0.01$) 7, 14, and 21 days following the first exposure; by mid-dose males and females ($p < 0.01$), but

only at study day 7; and by low-dose males ($p < 0.05$), but not females, 21 days after the first exposure (Report Table 7; Appendix E). Effects at the high dose were considered definitively exposure-related, while those at lower doses only suggestively so.

3. Clinical Laboratory Evaluations

a. Cholinesterase Activities - Significant dose-related inhibition of erythrocyte, plasma, and brain enzyme activities was found at all test exposures, with RBC cholinesterase being the most severely affected (Report Tables 8 through 13; Appendices F, G, and H).

b. Hematology (Report Table 12; Appendix I) - The only statistically significant differences from controls recorded were decreased platelet count in high-dose males ($p < 0.01$) and decreased MCV in high-dose females ($p < 0.01$), both of which the authors did not consider exposure-related.

c. Serum Chemistry (Report Table 15; Appendix K) - A number of statistically significant differences from concurrent controls were found (listed below), none of which were considered exposure-related by the study authors, but rather "within normal biological limits."

[NB: The report does not include documentation nor background support for this assertion.]

Parameter	Group	Sex	Change	Significance
Glucose	Low	F	Increase	$p < 0.05$
Phosphorus	Mid	M	Increase	$p < 0.05$
	Mid	F	Increase	$p < 0.05$
	High	F	Increase	$p < 0.01$
Cholesterol	High	M	Increase	$p < 0.05$
Globulin	High	F	Decrease	$p < 0.01$
Total Protein	High	F	Decrease	$p < 0.01$

d. Pathology - No exposure-related gross changes were recorded (Appendix M). Microscopic lesions, however, were observed in the nasal epithelium of both sexes treated at the mid and high concentrations, as well as in males at the low concentration. These lesions were characterized as squamous metaplasia, and were

most severe in high-dose animals (10/10 males, 8/10 females), and accompanied by acute rhinitis in 7 males and 2 females. At the mid-dose, milder forms of metaplastic changes were recorded in 4 males and 2 females, while minimal involvement was reported in the 2 low-dose males.

Other histopathological changes found in a few mid- and high-dose animals (conjunctival calcification, corneal dystrophy, mineralization, keratitis) were considered of "doubtful toxicological significance," but rather representing "residual lesions associated with bacterial or viral infection" [however, no definitive documentation was offered in support].

e) Organ Weights (Report Table 16; Appendix N) - Statistically significant differences in mean organ values between control and test groups were found in:

- Low-dose males: Increased left kidney weight; Increased left kidney/brain weight ratio; and Increased left testes/body weight ratio. [The authors suggest these may not be exposure-related.]

- Mid-dose males: Increased left kidney/body weight ratio.

- Mid-dose females: Increased brain/body weight ratio, and Increased right kidney/body weight ratio. [These were considered as "probably" exposure-related.]

- High-dose males: Decreased right/left testes weights; Increased right/left adrenal weights; Increased brain/body weight ratio; Increased liver weight; and Increased left kidney/body weight ratio.

- High-dose females: Increased brain/body weight ratio;
  Increased lungs/body weight ratio;
  Increased liver weight;
  Increased liver/brain weight ratio;
  Increased left kidney/body weight ratios; and
  Increased right kidney/body weight ratio.
  [These changes were considered exposure-related.]

The authors concluded that the nasal and corneal effects at the mid and high doses probably resulted from the corrosive irritant property of the naled aerosol.* Minimal epithelial effects, however, were also acknowledged at the LDT. Inhibition of both circulating and tissue-bound ChE enzyme activities occurred at all exposure concentrations. Finally, it was affirmed that the results of this 3-week study were to be used to select doses for the subsequent 13-week inhalation assay (SOCAL 2400).**

TB Evaluation:

Since this study was a rangefinder, without a NOEL, it has been judged CORE-SUPPLEMENTARY DATA.

[Although a statement affirming the study was inspected for compliance with EPA's GLP, no formal QA statement was included in the Final report.]

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*Toxicity Category I for eye effects (Naled Registration Standard).

**Already reviewed by TB, Document No. 005784, dated March 17, 1987, and judged CORE-MINIMUM DATA.